09/20/JC20 Rec'd PCT/PTO SEP 18 2001

FORM PTO-1390 (Modified) (REV 11-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	
01-1081	
U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)	
09/937103	

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/FR00/00730	23 March 2000	23 March 1999

TITLE OF INVENTION
USE OF TREHALOSE FOR STABILIZING A LIQUID VACCINE

APPLICANT(S) FOR DO/EO/US
1) Sandrine Lentsch Grai 2)Jean-Rene Cartier

OIPE JC20 SEP 18 2001 PATENT & TRADEMARK OFFICE

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (24) indicated below.
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S C. 371 (c) (2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☒ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☒ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made, however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. ☐ An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☐ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☐ A copy of the International Search Report (PCT/ISA/210).

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☒ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
20. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
21. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
22. ☐ Certificate of Mailing by Express Mail
23. ☒ Other items or information:

Patent Application Data Sheet
Return Postcard
Clean and Redlined Version of Amended Claims

PCTUS1/REV03

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
09/937103	PCT/FR00/00730	01-1081

24. The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

		CALCULATIONS PTO USE ONLY
☐	Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO	$1000.00
☒	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO	$860.00
☐	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	$710.00
☐	International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4)	$690.00
☐	International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4)	$100.00

ENTER APPROPRIATE BASIC FEE AMOUNT = $860.00

Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)). $0.00

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	10 - 20 =	0	x $18.00	$0.00
Independent claims	2 - 3 =	0	x $80.00	$0.00
Multiple Dependent Claims (check if applicable).			☐	$0.00

TOTAL OF ABOVE CALCULATIONS = $860.00

☐ Applicant claims small entity status. (See 37 CFR 1.27). The fees indicated above are reduced by 1/2. $0.00

SUBTOTAL = $860.00

Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). + $0.00

TOTAL NATIONAL FEE = $860.00

Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) (check if applicable). ☒ $40.00

TOTAL FEES ENCLOSED = $900.00

Amount to be: refunded $

charged $

a. ☒ A check in the amount of **$900.00** to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **13-2490**. A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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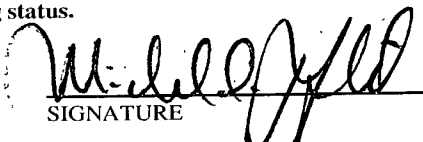

SIGNATURE

Michael S. Greenfield

NAME

34,172

REGISTRATION NUMBER

18 September 2001

DATE

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
(Case No. 00-1081)

In the Application of:)
)
Lentsch Graf, *et al.*)
) **Examiner: TBA**
Serial No.: U.S. Nat'l Phase of PCT/FR00/00730)
) **Group Art Unit: TBA**
Filing Date: March 23, 2000)
)
For: Use of Trehalose for Stabilizing a Liquid Vaccine)

PRELIMINARY AMENDMENT

Asst. Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Please consider the following amendments and remarks before examination on the merits.

AMENDMENTS

In the claims:

Please amend the claims as follows:

1. (Amended) A liquid vaccine composition comprising at least one antigen consisting of a polysaccharide bound to a carrier protein, wherein it additionally comprises trehalose.

2. (Amended) The vaccine composition as claimed in claim 1, wherein said polysaccharaide is the capsular polysaccharide of Haemophilus influenzae type b or Polyribosylribitol Phosphate.

3. (Amended) The vaccine composition as claimed in claim 1, wherein said polysaccharide is a pneumococcal polysaccharide.

4. (Amended) The vaccine composition as claimed in claim 1, wherein said polysaccharide is a meningococcal polysaccharide

5. (Amended) The vaccine composition as claimed in claim 1, wherein the said carrier protein is tetanus toxoid.

6. (Amended) The vaccine composition as claimed in claim 1, wherein said carrier protein is diphtheria toxoid.

7. (Amended) The vaccine composition as claimed in claim 1, wherein the quantity of trehalose is between 3 and 12% by mass.

8. (Amended) The vaccine composition as claimed in claim 1, wherein the quantity of trehalose is about 5%.

9. (Amended) The method of stabilizing a liquid vaccine composition comprising at least one antigen consisting of a polysaccharide bound to a carrier protein, wherein it consists in adding trehalose to the vaccine composition.

10. (Amended) The method as claimed in claim 9, wherein the quantity of trehalose to be added is between 3 and 12% by mass.

REMARKS

The foregoing amendments merely correct formal matters and remove all multiple dependencies in order to reduce the filing fee and bring the claims into conformance with U.S. practice by removing multiple dependent claims that depend from multiple dependent claims. No new subject matter has be introduced by way of these amendments. Clean and redlined versions of the amended and new claims accompany this submission.

If there are any questions or comments regarding this Preliminary Amendment or application, the Examiner is encouraged to contact the undersigned attorney as indicated below.

Respectfully submitted,

Date: September 18, 2001

Michael S. Greenfield
Registration No. 37,142

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Redlined Version of Amended Claims

1. (Amended)A liquid vaccine composition comprising at least one antigen consisting of a polysaccharide bound to a carrier protein, ~~characterized in that~~ wherein it additionally comprises trehalose.

2. (Amended)The vaccine composition as claimed in claim 1, ~~characterized in that~~ wherein said polysaccharaide is the capsular polysaccharide of Haemophilus influenzae type b or Polyribosylribitol Phosphate.

3. (Amended)The vaccine composition as claimed in claim 1, ~~characterized in that~~ wherein said polysaccharide is a pneumococcal polysaccharide.

4. (Amended)The vaccine composition as claimed in claim 1, ~~characterized in that~~ wherein said polysaccharide is a meningococcal polysaccharide

5. (Amended) The vaccine composition as claimed in ~~the preceding~~ claims1, ~~characterized in that~~ wherein the said carrier protein is tetanus toxoid.

6. (Amended)The vaccine composition as claimed in ~~the preceding~~ claims 1, ~~characterized in that~~ wherein said carrier protein is diphtheria toxoid.

7. (Amended)The vaccine composition as claimed in ~~the preceding~~ claims 1, ~~characterized in that~~ wherein the quantity of trehalose is between 3 and 12% by mass.

8. (Amended)The vaccine composition as claimed in ~~the preceding~~ claims 1, ~~characterized in that~~ wherein the quantity of trehalose is about 5%.

9. (Amended)The method of stabilizing a liquid vaccine composition comprising at least one antigen consisting of a polysaccharide bound to a carrier protein, ~~characterized in that~~ wherein it consists in adding trehalose to the vaccine composition.

10. (Amended)The method as claimed in claim 9, ~~characterized in that~~ wherein the quantity of trehalose to be added is between 3 and 12% by mass.

Clean Version of Amended Claims

1. A liquid vaccine composition comprising at least one antigen consisting of a polysaccharide bound to a carrier protein, wherein it additionally comprises trehalose.

2. The vaccine composition as claimed in claim 1, wherein said polysaccharaide is the capsular polysaccharide of Haemophilus influenzae type b or Polyribosylribitol Phosphate.

3. The vaccine composition as claimed in claim 1, wherein said polysaccharide is a pneumococcal polysaccharide.

4. The vaccine composition as claimed in claim 1, wherein said polysaccharide is a meningococcal polysaccharide

5. The vaccine composition as claimed in claim 1, wherein the said carrier protein is tetanus toxoid.

6. The vaccine composition as claimed in claim 1, wherein said carrier protein is diphtheria toxoid.

7. The vaccine composition as claimed in claim 1, wherein the quantity of trehalose is between 3 and 12% by mass.

8. The vaccine composition as claimed in claim 1, wherein the quantity of trehalose is about 5%.

9. The method of stabilizing a liquid vaccine composition comprising at least one antigen consisting of a polysaccharide bound to a carrier protein, wherein it consists in adding trehalose to the vaccine composition.

10. The method as claimed in claim 9, wherein the quantity of trehalose to be added is between 3 and 12% by mass.

USE OF TREHALOSE FOR STABILIZING A LIQUID VACCINE

The invention relates to the field of vaccines. More particularly, the invention relates to liquid vaccine compositions comprising, among their antigens, at least one polysaccharide bound to a carrier protein.

Such vaccine compositions, some of whose antigens have to be bound to carrier proteins in order to be immunogenic, are known in the prior art. This is in particular the case for compositions intended for vaccination against infections caused by the bacterium Haemophilus influenzae type b, which comprise, as vaccine antigen, the capsular polysaccharide of the bacterium or Polyribosylribitol Phosphate (PRP) coupled to the tetanus toxoid T. Such vaccine compositions tend to lose their immunogenicity, and therefore their efficacy, over time. To overcome this drawback, the solution generally proposed in the prior art is freeze-drying. This solution, which is satisfactory from the point of view of the result obtained as regards preservation of immunogenicity, has, nevertheless, the disadvantage of making the method of manufacture cumbersome, and therefore of increasing the cost thereof. In addition, at the time of administration of the vaccine, it is necessary to carry out an additional operation of taking up the freeze-dried product in a sterile liquid, which, on the one hand, represents an additional constraint for the practitioner and, on the other hand, comprises, like any manipulation, the risk of being poorly carried out. It is therefore desirable to find another solution to the problem of the loss of immunogenicity, over time, of the polysaccharide antigens bound to a carrier protein when they are present in a liquid vaccine composition.

To this end, the invention provides a liquid vaccine composition comprising at least one antigen consisting

of a polysaccharide bound to a carrier protein, characterized in that it additionally comprises trehalose.

Thus, a vaccine composition is obtained which, although liquid, preserves it immunogenic character over time, even when it is stored at room temperature.

The subject of the present invention is also a method of stabilizing a liquid vaccine composition comprising at least one antigen consisting of a polysaccharide bound to a carrier protein, characterized in that it consists in adding trehalose to the vaccine composition.

The method according to the invention has the advantage of being simple and fast, which makes it a method of choice for a manufacturer.

Numerous other advantages of the present invention will emerge on reading the detailed description which follows.

The vaccine composition according to the invention may be a monovalent composition, that is to say that it is intended for protection against a single disease, or a multivalent composition, that is to say that it is intended to protect the individual to whom it has been administered, against several diseases. In all cases, at least one of the vaccine valencies is represented by a polysaccharide antigen bound to a carrier protein. Among the polysaccharide antigens capable of entering into the composition of a vaccine and of being stabilized according to the invention, there may be mentioned the polysaccharides present in the capsules of bacteria, the polysaccharides present in the walls of Gram-negative bacteria or the polysaccharides present in the walls of fungi. Thus, it is possible to use the polysaccharides encountered in the following

microorganisms: Pseudomonas (for example P. aeruginosa), Saphylococcus, Steptococcus (for example S. pneumoniae), Klebsiella (for example K. pneumonia), Salmonella (for example S. typhi and S. paratyphi), Escherichia coli, Neisseria (for example N. meningitidis), Shigella (for example S. dysenteria, somnei or flexneri), Haemophilus (for example H. influenzae type b), Moraxella, Vibrio cholerae, Mycobacterium tuberculosis, Candida, Cryptococcus neoformans and Hansenula.

The present invention has shown all its benefit for vaccine compositions comprising the capsular polysaccharide of Haemophilus influenzae type b or Polyribosylribitol Phosphate.

The polysaccharides generally used as vaccine antigens generally exhibit the characteristic of being T-independent, that is to say in particular that the memory of the immune system in relation to such antigens is weak and that these polysaccharides are generally not immunogenic in young children. To make them T-dependent, it is customary to combine them with carrier proteins (protein, for the purposes of the present invention, also includes peptides or polypeptides) in order to obtain a polysaccharide-carrier protein conjugate. These proteins are in particular those normally used in the field of vaccines: diphtheria toxoid, tetanus toxoid, nontoxic mutant form CRM_{197} of diphtheria toxoid, outer membrane protein type 1 (OMP1) of Neisseria meningitidis, as well as any native or synthetic peptide or polypeptide capable of fulfilling the same function, for example the peptides described in patent application WO98/31393.

The binding of the polysaccharide to the carrier protein can vary according to the polysaccharide and the protein used. It generally involves covalent

bonding, which can call into play a spacer arm. According to the mode of binding used, the antigen obtained, which is generally called conjugate, is an antigen in which the polysaccharide is bound to the carrier protein by a single chemical functional group (conjugates of the sun or neoglycoconjugate type) or by several functional groups (conjugates of the scraper or coil type).

As the vaccine composition according to the invention may be multivalent, it is possible to add to the antigen consisting of the polysaccharide-carrier protein conjugate one or more other valencies also consisting of a polysaccharide-carrier protein conjugate, or of any other different type of antigen. Among the other valencies which may enter into the vaccine composition according to the invention, there may be mentioned in particular: whooping cough, polio, diphtheria, tetanus, hepatitis (A, B, C and the like), varicella, mumps, measles, Dengue, Japanese encephalitis, yellow fever, rubella, influenza, meningitis, pneumonia, and the like.

The vaccine composition according to the invention may comprise, in addition, all the components usually present in a vaccine: buffer or physiological saline, preservative and one or more adjuvants.

According to a characteristic of the invention, this vaccine composition comprises, in addition, trehalose in a sufficient quantity to allow the immunogenicity of the antigen consisting of the polysaccharide conjugate to be maintained over time.

Trehalose or α-D-glucopyranosyl α-D-glucopyranoside is a disaccharide known for its protective action in relation to proteins when they are subjected to high temperatures, in particular during drying or freeze-drying operations. According to the teaching of

document US 4 891 319, its protective action may be explained by a replacement of the water molecules by trehalose molecules, the 2 compounds comprising OH functional groups.

Trehalose is also known in the prior art as a cell protectant.

Surprisingly, and without this being deducible from the known properties of trehalose, it has now been found that this compound makes it possible to preserve the immunogenicity of vaccine compositions, even in the case where the latter might not be subjected to a rise in temperature or to a drying process.

On the other hand, other sugars tested which are known to have properties similar to trehalose, in particular lactose, did not lead to satisfactory results.

According to a particular characteristic of the invention, it has been observed that a quantity of trehalose of between 3 and 12%, and preferably 5, was satisfactory to solve the problem of stability of the vaccine composition. At this concentration, no toxicity reaction was revealed.

The trehalose suitable for the purposes of the invention should be a trehalose of pharmaceutical quality, without it being necessary, nevertheless, for it to have an absolute degree of purity. The trehalose provided by the company SIGMA under the reference T9531 is perfectly suitable.

The trehalose may be added at the beginning of the method of manufacture; it may also be added to the formulation at the end of the method, alone or in the form of a mixture with other excipients.

The following examples illustrate more particularly one embodiment of the invention.

Example 1

Three different vaccine compositions are prepared by proceeding in the following manner:

a- Manufacture of 4 stock solutions of excipients

- 50 mM solution of Tris Hydroxyl Amino Methane - 42.5% sucrose

Composition for 1 liter:
6.06 g Tris Hydroxyl Amino Methane
425 g sucrose
Water for injection qs 1 liter

- Solution of trehalose at 20%

Composition for 400 ml
Trehalose: 80 g
Water for injection qs 400 ml
pH = 7 ± 0.1 after adjusting with a 2.5N sodium hydroxide solution

- 2M solution of sodium chloride

Composition for 1 liter
Sodium chloride: 117 g
Water for injection qs 1 liter

- 50 mM Tris Hydroxyl Amino Methane

Composition for 1 liter
Tris Hydroxyl Amino Methane: 6.06 g
5N HCl: 8.54 ml
Water for injection qs 1 liter

b- Production of 3 solutions of excipients (A, B, C) from the preceding 4 stock solutions. The volumes of the stock solutions used are indicated in the table below:

Stock solutions	Excipient 1	Excipient 2	Excipient 3
50 mM Tris Hydroxyl Amino Methane solution, 42.5% sucrose	72.6 ml	0	0
20% trehalose solution	0	100 ml	200 ml
2M sodium chloride solution	0	19 ml	0
50 mM Tris Hydroxyl Amino Methane solution	0	72.6 ml	72.6 ml
Water for injection	qs 363 ml	qs 363 ml	qs 363 ml

c- Each solution of excipient is sterilized by filtration on a Millipack 60 filter having a cut-off of 0.22 µm.

d- To obtain each of the vaccine compositions, there are added to a 500 ml Schott flask, sterilized by autoclaving, in the order: 290 ml of each of the solutions of excipients prepared and then 29.6 ml of a composition containing PRP-T and sucrose. The flasks are stirred for 5 minutes at room temperature and then for 2 hours at 4°C.

e- Each composition is distributed into glass serum bottles which are kept for 6 months at 25°C.

The final composition of each formulation is summarized in the following table:

	Composition A	Composition B	Composition C`
PRP-T (µg of polysaccharide/ml)	20	20	20
Tris Hydroxyl Amino Methane (mM)	10 mM	10 mM	10 mM
Sucrose (%)	8.5%	0.78%	0.78%
Trehalose (%)	0%	5%	10%
NaCl (mM)	0	0.095 mM	0

Example 2

Five groups of 8 female OF 1 mice, weighing 22 to 24 grams, are available. The mice are divided into groups of 8. Each group is used to test one of the vaccine compositions A, B or C obtained in example 1, a vaccine composition serving as negative control (comprising only nonconjugated PRP) and a vaccine composition serving as positive control which consists of the vaccine Act-Hib™ marketed by the company PASTEUR MERIEUX Sérum and Vaccins.

0.5 ml of the vaccine composition to be tested, corresponding to 2.5 µg of polysaccharide, is administered to each mouse, by the subcutaneous route. Each mouse receives one injection at D0 and one booster injection at D14.

The serum of each mouse is collected at D0, D14 and D21.

Example 3

The sera collected are assayed by RadioImmunoAssay. The results obtained are exploited in the following manner:

- The geometric mean is calculated from the titer of 8 sera.
- The % of responsive mice (serum having a titer > 0.5) is determined.
- The difference between the titers obtained at D14 and D21 is calculated so as to evaluate the effect of the booster injection.

The product is declared to be in conformity when the following 3 conditions are met:

- At D21, at least 75% of the mice have a titer >=0.5.
- The difference between the titers obtained at D14 and D21 is statistically significant.

- The difference in titer between the product tested and the positive control is not statistically significant at D21.

The results obtained are summarized in the following table:

	GMT at D14	**GMT at D21**	**Conformity of the product**
Negative control	< 0.09	0.05	-
Positive control	< 0.1	1.3	+
Composition A	0.33	0.99	-
Composition B	0.13	1.6	+
Composition C	0.11	2.9	+

These results show that the vaccine compositions according to the invention preserve their immunogenicity after storage for 6 months at 25°C.

Tests carried out in the same manner on compositions stored at 37°C showed that a composition according to the invention, comprising 5% trehalose, retained its immunogenic character even after 3 months of storage.

CLAIMS

1. A liquid vaccine composition comprising at least one antigen consisting of a polysaccharide bound to a carrier protein, characterized in that it additionally comprises trehalose.

2. The vaccine composition as claimed in claim 1, characterized in that said polysaccharide is the capsular polysaccharide of Haemophilus influenzae type b or Polyribosylribitol Phosphate.

3. The vaccine composition as claimed in claim 1, characterized in that said polysaccharide is a pneumococcal polysaccharide.

4. The vaccine composition as claimed in claim 1, characterized in that said polysaccharide is a meningococcal polysaccharide.

5. The vaccine composition as claimed in one of the preceding claims, characterized in that said carrier protein is tetanus toxoid.

6. The vaccine composition as claimed in one of the preceding claims, characterized in that said carrier protein is diphtheria toxoid.

7. The vaccine composition as claimed in one of the preceding claims characterized in that the quantity of trehalose is between 3 and 12% by mass.

8. The vaccine composition as claimed in the preceding claims, characterized in that the quantity of trehalose is about 5%.

9. A method of stabilizing a liquid vaccine composition comprising at least one antigen consisting of a polysaccharide bound to a carrier protein, characterized in that it consists in adding trehalose to the vaccine composition.

10. The method as claimed in claim 9, characterized in that the quantity of trehalose to be added is between 3 and 12% by mass.

PCT ORGANISATION MONDIALE DE LA PROPRIETE INTELLECTUELLE
Bureau international

DEMANDE INTERNATIONALE PUBLIEE EN VERTU DU TRAITE DE COOPERATION EN MATIERE DE BREVETS (PCT)

(51) Classification internationale des brevets 7 : A61K 47/26, 39/02, 39/09, 39/095, 39/102, 39/385, A61P 31/04

A1

(11) Numéro de publication internationale: WO 00/56365

(43) Date de publication internationale: 28 septembre 2000 (28.09.00)

(21) Numéro de la demande internationale: PCT/FR00/00730

(22) Date de dépôt international: 23 mars 2000 (23.03.00)

(30) Données relatives à la priorité:
99/03765 23 mars 1999 (23.03.99) FR

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(72) Inventeurs; et

(75) Inventeurs/Déposants *(US seulement)*: LENTSCH GRAF, Sandrine [FR/FR]; 10, rue Janin, F–69004 Lyon (FR). CARTIER, Jean–René [FR/FR]; 55, rue Joliot Curie, F–69005 Lyon (FR).

(74) Mandataire: KERNEIS, Danièle; Direction de la Propriété Industrielle, Aventis Pasteur, 2, avenue Pont Pasteur, F–69007 Lyon (FR).

(81) Etats désignés: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, brevet ARIPO (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), brevet eurasien (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), brevet européen (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), brevet OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Publiée
Avec rapport de recherche internationale.

(54) Title: USE OF TREHALOSE FOR STABILISING A LIQUID VACCINE

(54) Titre: UTILISATION DE TREHALOSE POUR STABILISER UN VACCIN LIQUIDE

(57) Abstract

The invention concerns a liquid vaccine composition comprising at least an antigen consisting of a polysaccharide bound to a carrier protein and trehalose. The invention also concerns a method for stabilising a liquid vaccine composition which consists in adding trehalose to the vaccine composition.

(57) Abrégé

L'invention concerne une composition vaccinale liquide comprenant au moins un antigène constitué par un polysaccharide lié à une protéine porteuse, ainsi que du tréhalose. L'invention concerne également un procédé de stabilisation d'une composition vaccinale liquide selon lequel on ajoute à la composition vaccinale du tréhalose.

Case No.: 01-1081

DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Use of Trehalose for Stabilising A Liquid Vaccine

the specification of which is the same as **PCT/FR00/00730**.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR § 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT international application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s):

	Number	Country	Day/Month/Year Filed
1.	**99/03765**	**France**	**23 March 1999**
2.			

I hereby claim the benefit under 35 U.S.C. § 119(e) of any United States provisional application(s) listed below:

	Application Number	Filing Date
1.		
2.		

I hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s), or § 365(c) of any PCT international application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 C.F.R. § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

	Application Number	Filing Date
1.	**PCT/FR00/00730**	**23 March 1999**
2.		

I hereby appoint the practitioners associated with the Customer Number provided below to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith, and I direct that all correspondence be addressed to that Customer Number.

Customer Number: **020306**
Principal attorney or agent: **Michael S. Greenfield**
Telephone number: **312-913-0001**

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Customer Number: **020306**
Principal attorney or agent: **Michael S. Greenfield**
Telephone number: **312-913-0001**

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1 – 00

Full name of first inventor: **Sandrine Lentsch Graf**

Inventor's signature: Sandrine Lentsch-Graf Date: 1 September 2001
Residence: **10, rue Janin, F-69004 Lyon, France**
Citizenship: **A Citizen of France** FRX
Post Office Address: **10, rue Janin, F-69004 Lyon, France**

Full name of second inventor: **Jean René Cartier (deceased)**

Date: _____

Signed by: _____, Legal Representative of the Estate of Jean René Cartier

Inventor's Residence: **55, rue Juliot Curie, F-69005 Lyon, France**
Inventor's Citizenship: **A Citizen of France**
Inventor's Post Office Address: **55, rue Juliot Curie, F-69005 Lyon, France**

Representative's Residence: _____

Representative's Citizenship: **A Citizen of** _____

Representative's Post Office Address: _____

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Case No.: 01-1081

DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Use of Trehalose for Stabilising A Liquid Vaccine

the specification of which is the same as **PCT/FR00/00730**.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of first inventor: **Sandrine Lentsch Graf**

Inventor's signature: _____ Date: _____
Residence: **10, rue Janin, F-69004 Lyon, France**
Citizenship: **A Citizen of France**
Post Office Address: **10, rue Janin, F-69004 Lyon, France**

2-01
Full name of second inventor: **Jean René Cartier (deceased)**

Date: X 31. Mai 2002

Signed by: X _____, Legal Representative of the Estate of Jean René Cartier

3-11
Name: Claudine CARTIER
(typed or printed)

Inventor's Residence: **55, rue Juliot Curie, F-69005 Lyon, France** FRX
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